

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Hiroyuki Tsunoda *et al.*                      Art Unit : 1633  
Serial No. : 10/581,183                                      Examiner : Fereydoun Ghotb Sajjadi  
Filed : May 18, 2007                                        Conf. No. : 1638  
Title : EXPRESSION SYSTEMS USING MAMMALIAN BETA-ACTIN  
PROMOTER

**Mail Stop Amendment**  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**Declaration of Hiroyuki Tsunoda Under 37 C.F.R. § 1.132**

I, the undersigned, Hiroyuki Tsunoda, residing at 153-2 Nagai, Niihari-mura, Niihari-gun, Ibaraki, JAPAN, declare and state as follows:

1. I am a co-inventor named on U.S. Patent Application No. 10/581,183 ("the Application").
2. My curriculum vitae is attached as Exhibit A.
3. I have reviewed and am familiar with the Office Action dated August 14, 2009 ("the Office Action"), issued by the U.S. Patent and Trademark Office in the Application.
4. I have reviewed and am familiar with the Application's specification, filed on May 18, 2007, and currently pending claims.
5. Exhibit B is a graph illustrating the results of an experiment that was conducted under my direction and control, using the experimental procedures generally described in Example 2 of the Application. In this experiment, the activities of various promoters (mouse  $\beta$  actin promoter, chicken  $\beta$  actin promoter, and human EF1 $\alpha$  promoter), alone or in combination with human CMV enhancer and in one case WPRE, were compared using a luciferase reporter gene assay. Specifically, the promoter (with or without human CMV and/or WPRE) was cloned into the multiple cloning site of pGL3-Basic (Promega, Cat. No. E1751), and this vector was transiently expressed in DG44 cells. Luciferase activity was determined using the Luciferase Assay System (Promega, Cat. No. E1501). After removing the medium, 100  $\mu$ l of passive lysis buffer was added to each well, and the cells were lysed by shaking. 10  $\mu$ l of the cell lysate was transferred to Assay Plate Tissue Culture Treated White with Clear Bottom (Corning, Cat No. 437842). The measurement was performed using MicroLumat (Berthold), and the data were collected using the WinGlow-Control Program LB96PV software, ver.1.24.

**CERTIFICATE OF MAILING BY EFS-WEB FILING**

I hereby certify that this paper was filed with the Patent and Trademark Office using the EFS-WEB system on this date: Feb. 16, 2010